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Interleukin-27 induces the endothelial differentiation in Sca-1+ cardiac resident stem cells

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ABSTRACT

Cytokines play important roles in cardiac repair and regeneration. Recently, we demonstrated that interleukin (IL)-6 family cytokines induce the endothelial differentiation of Sca-1+ cardiac resident stem cells through STAT3/Pim-1 signaling pathway. In contrast, the biological functions of IL-12 family cytokines in heart remain to be elucidated, though they show structural homology with IL-6. In the present study, we examined the effects of IL-12 family cytokines on the transdifferentiation of cardiac Sca-1+ cells into cardiac cells. RT-PCR analyses revealed that IL-27 receptor α (IL-27Rα), but not IL-12R or IL-23R, was expressed in cardiac Sca-1+ cells. The transcript expression of IL-27 was elevated in murine hearts in cardiac injury models. Intriguingly, IL-27 stimulation for 14 days induced the endothelial cell (EC) marker genes, such as *CD-31* and *VE-cadherin*. Immunoblot analyses clarified that IL-27 treatment rapidly phosphorylated STAT3. IL-27 upregulated the expression of Pim-1, but the overexpression of dominant negative STAT3 abrogated the induction of Pim-1 by IL-27. Finally, adenoviral transfection of dominant negative Pim-1 inhibited IL-27-induced EC differentiation of cardiac Sca-1+ cells. These findings demonstrated that IL-27 promoted the commitment of cardiac stem cells into the EC lineage, possibly leading to neovascularization as a novel biological function. IL-27 could not only regulate the inflammation but also contribute to the maintenance of the tissue homeostasis through stem cell differentiation at inflammatory sites.

1. Introduction

Accumulating evidence has revealed that IL-6 family cytokines play important roles in vascular formation. IL-6 cytokines bind to their specific receptor and gp130, followed by STAT3 activation. The activation of STAT3 induces angiogenic factors, such as VEGF, increasing capillary vessels [1] in the normal or cancer tissues [2–5]. In addition, we have recently demonstrated that IL-6 family cytokines induce endothelial differentiation of Sca-1+ cardiac resident stem cells [6,7], suggesting that IL-6 family cytokines contribute to neovascularization not only by stimulating pre-existing ECs but also by regulating commitment of tissue resident stem cells into ECs. In Sca-1+ cardiac resident stem cells, the activation of STAT3 is essential for the EC differentiation induced by IL-6 family cytokines. Activated STAT3 rapidly upregulates the expression of Pim-1 serine/threonine kinase as a critical event for the transdifferentiation [8]. Importantly, the transplantation of cardiac Sca-1+ cells in post-infarct myocardium resulted in the increased capillary density, while not the Sca-1+ cells transfected with dominant negative Pim-1.

Recently, much attention has been paid to IL-12 family cytokines as regulators of inflammation [9,10]. IL-12 cytokine family, which is composed of IL-12, IL-23, IL-27, and IL-35, is evolutionarily linked to the IL-6 cytokine superfamily. The members of the IL-12 family are heterodimeric proteins consisting of alpha and beta chains. The alpha chains, such as p19, p28, and p35, share structural homology with IL-6, whereas the beta chains, p40 or Epstein–Barr virus-induced gene 3 (EBI3), with soluble cytokine receptor chains, such as IL-6Rα. These cytokines transduce signals through unique heterodimeric receptors, leading to the activation of JAK-STAT pathways. Interestingly, it was reported that IL-27Rα, also designated as WSX-1, is highly expressed in the heart, though its biological function remains to be addressed [11].

In the present study, we examined the functional significances of IL-27/IL-27Rα by focusing on cardiac resident stem cells. Here, we found that IL-27 induced the endothelial differentiation in cardiac Sca-1+ cells as a novel biological function. This is the first

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demonstration that IL-27 regulates the cell fate of cardiac resident stem cells.

2. Materials and method

2.1. Animal experiments

The animal experiments were approved by the Institutional Animal Care and Use Committee of Graduate School of Pharmaceutical Sciences, Osaka University (Permit Number; DOUYAKU 19-32-6). The investigation is compliance with the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (National Institutes of Health publication No. 85-23. revised 1996).

2.2. Preparation of cardiac Sca-1+ cells and cytokine treatment

Cardiac Sca-1+ cells were isolated by magnetic cell sorting (MACS) from wild type C57Bl/6 mice (8–10 weeks old, Japan SLC) with about 98% purity, as previously described [6].

Recombinant mouse IL-27 (#2799-ML-010) was purchased from R&D systems. Isolated cardiac Sca-1+ cells were treated with IL-27 at different concentrations for the indicated time.

2.3. Induction of myocardial infarction and experimental autoimmune myocarditis

Myocardial infarction (MI) was generated by ligation of the left anterior descending coronary artery according to previous report with minor modifications [12]. In brief, C57/Bl6 mice were anesthetized and ventilated with 100% oxygen containing 1.5% isoflurane. After left-side thoracotomy, the left coronary artery was ligated with 7-0 silk sutures. The chest and the skin were closed with 5-0 silk sutures. At days 0, 4, 7 and 14 after MI, mice were sacrificed, followed by analysis of gene expressions in hearts.

Experimental autoimmune myocarditis (EAM) was induced as described previously [13]. Briefly, BALB/c mice were immunized with peptides derived from the sequence of the murine α-cardiac myosin heavy chain (Myhc). Myhc-α peptides, diluted in PBS, were emulsified in complete Freund's adjuvant. On days 0 and 7, 100 μg of Myhc-α peptides in 200 μL of the emulsion was subcutaneously treated. At days 0, 14, 21 and 28 after first immunization, gene expressions in hearts were measured by quantitative RT-PCR, as described below.

2.4. RT-PCR analyses/quantitative RT-PCR analyses

RT-PCR was performed as previously described [6]. Briefly, total RNA was prepared from cardiac Sca-1+ cells with acid guanidinium thiocyanate-phenol-chloroform method. Total RNA (1 μg) was subjected for the first strand cDNA synthesis by using the oligo (dT) first strand primer. Gene-specific primers used for PCR amplification were shown in Table 1. The PCR products were size-fractionated by 2% agarose gel electrophoresis.

After synthesis of the first strand cDNA, quantitative PCR was performed with the use of Applied Biosystems StepOne™ Real-Time PCR System (Applied Biosystems, CA) with the FAST SYBR Green PCR Master Mix (Applied Biosystems, CA). GAPDH was used as an internal control.

2.5. Immunoblot analyses

Immunoblot analyses were performed as described previously [6]. Cell lysates were prepared by adding SDS-PAGE sample solution to cells and boiled for 5 min. Proteins were separated by SDS-PAGE on polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore). The membrane was blocked with 2% skim milk for 1 h, followed by incubation with anti-phospho-STAT3 (#9131, Cell Signaling Technology), anti-phospho-ERK1/2 (#9101, Cell Signaling Technology), or anti-Pim-1 (#3247, Cell Signaling Technology) antibody, as a primary antibody, overnight at 4 °C. Horseradish peroxidase (HRP)-conjugated antibody (Santa Cruz Biotechnology) was used as secondary antibody. The bands were detected by ECL system (GE Healthcare). Densitometric analyses of the detected bands were performed with Image-J software. The membrane was reprobed with anti-STAT3 antibody (sc-7179, Santa Cruz Biotechnology), anti-ERK1/2 (#9102, Cell Signaling Technology), or anti-GAPDH antibody (MAB374, Millipore) to show equal amount of protein loading.

Table 1
The primers used in this study.

Genes	Direction	Sequence
VE-cadherin	Forward	5′-ATCTTCCTCTGCATCCTCAC-3′
	Reverse	5′-GTAAGTGACCAACTGCTCGT-3′
CD31	Forward	5′-GAGCCCAATCACGTTTCAGTTT-3′
	Reverse	5′-TCCTTCCTGCTTCTTGCTAGCT-3′
Flk-1	Forward	5′-TGCCGGCATGGTCTTCTGTGAGG-3′
	Reverse	5′-CATTGAGCTCTGTTCTCGCTGTAC-3′
Nkx-2.5	Forward	5′-CAGTGGAGCTGGACAAAGCC-3′
	Reverse	5′-TTGTAGCGACGGTTCTGGAA-3′
Calponin	Forward	5′-GCACATTTTAACCGAGGTCC-3′
	Reverse	5′-TGACCTTCTTCACAGAACCC-3′
IL-12Rβ1	Forward	5′-CAGGGACCAGCAAACACATC-3′
	Reverse	5′-TTCCTGGGTCCTAAGGGTGA-3′
IL-12Rβ2	Forward	5′-GACTCGACAGCACAACCTGA-3′
	Reverse	5′-TTGGGGACTTTCACCAGCAG-3′
IL-23R	Forward	5′-TTGGTATGGGTCCAAGCTGT-3′
	Reverse	5′-TCGTTTGTAGTCTCAGCCCT-3′
IL-27R	Forward	5′-AAGGGACCAGGAAACCGTTG-3′
	Reverse	5′-AATCCCCAAACTGAGGGTGC-3′
p28	Forward	5′-AGCCTGTTGCTGCTACCCTTGC-3′
	Reverse	5′-GTGGACATAGCCCTGAACCTCA-3′
EBI3	Forward	5′-TCTTCCTGTCACTTGCCCTCTG-3′
	Reverse	5′-AGTTGGGAGCCTGGAGAGGAGT-3′
GAPDH	Forward	5′-CATCACCATCTTCCAGGAGCG-3′
	Reverse	5′-GAGGGGCCATCCACAGTCTTC-3′

2.6. Immunofluorescent microscopic analyses

Immunofluorescent microscopic analyses were performed as described previously [14]. Briefly, cells were fixed with 3.7% formaldehyde in PBS for 20 min at room temperature, followed by staining with anti-Sca-1 (R&D Systems), IL-27Rα (Abcam) and anti-VE-cadherin antibodies (sc-6458, Santa Cruz Biotechnology). Alexa Fluor 488- or 546-conjugated secondary antibody (Molecular Probes) was used for detection. Nuclei were also stained with Hoechst 33258 (Sigma). Cells were examined with fluorescent microscopy (Olympus FSX100).

2.7. Construction and infection of adenovirus vectors

The construction of adenovirus vectors expressing dominant negative form of STAT3 (dn-STAT3), dominant-negative form of Pim-1 (dn-Pim-1), and β-galactosidase fused with a nuclear localization signal (β-gal) was previously described [2,8]. Sca-1+ cells were infected with adenovirus vectors at a multiplicity of infection

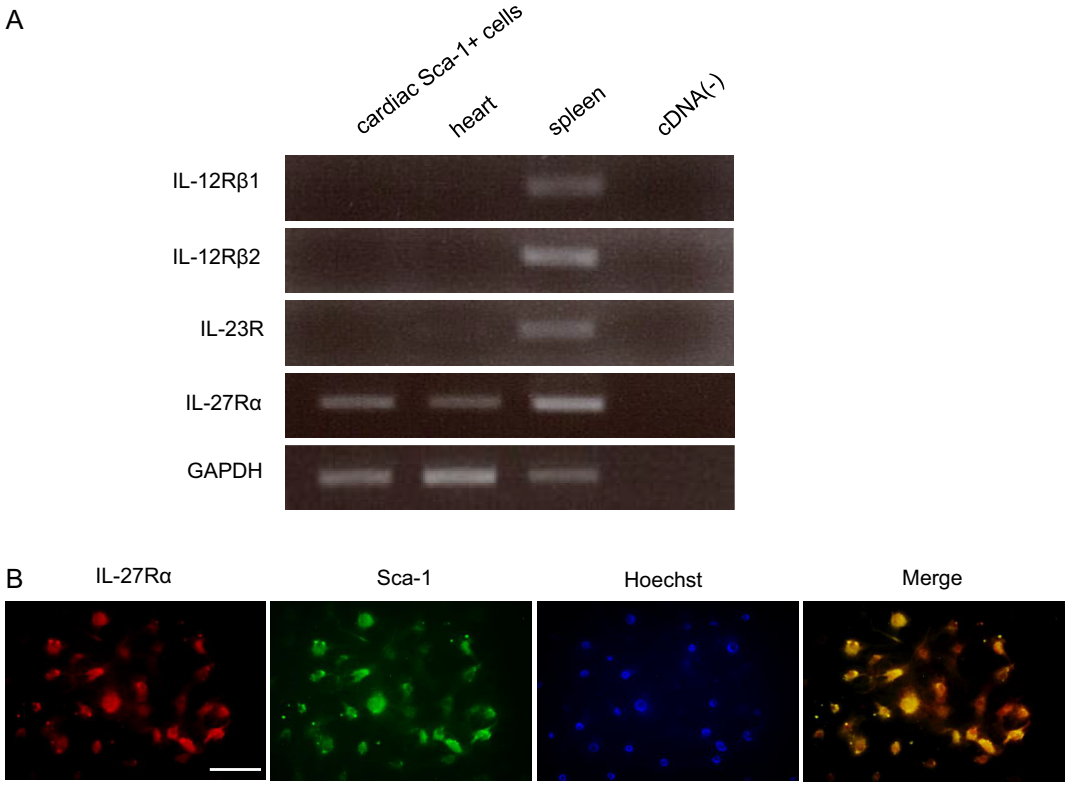

Fig. 1. IL-27Rα (WSX-1) was expressed in cardiac Sca-1+ cells. Cardiac Sca-1+ cells were isolated from C57Bl/6 mice. (A) The expressions of *IL-12Rβ1*, *IL-12Rβ2*, *IL-23R* and *IL-27Rα* (*WSX-1*) in Sca-1+ cells were examined by RT-PCR. Total RNA from murine spleen was used as a positive control. GAPDH was used as an internal control. (B) The expression of IL-27Rα protein was detected by immunofluorescent microscopic analysis. Representative images are shown. Bar = 100 μm.

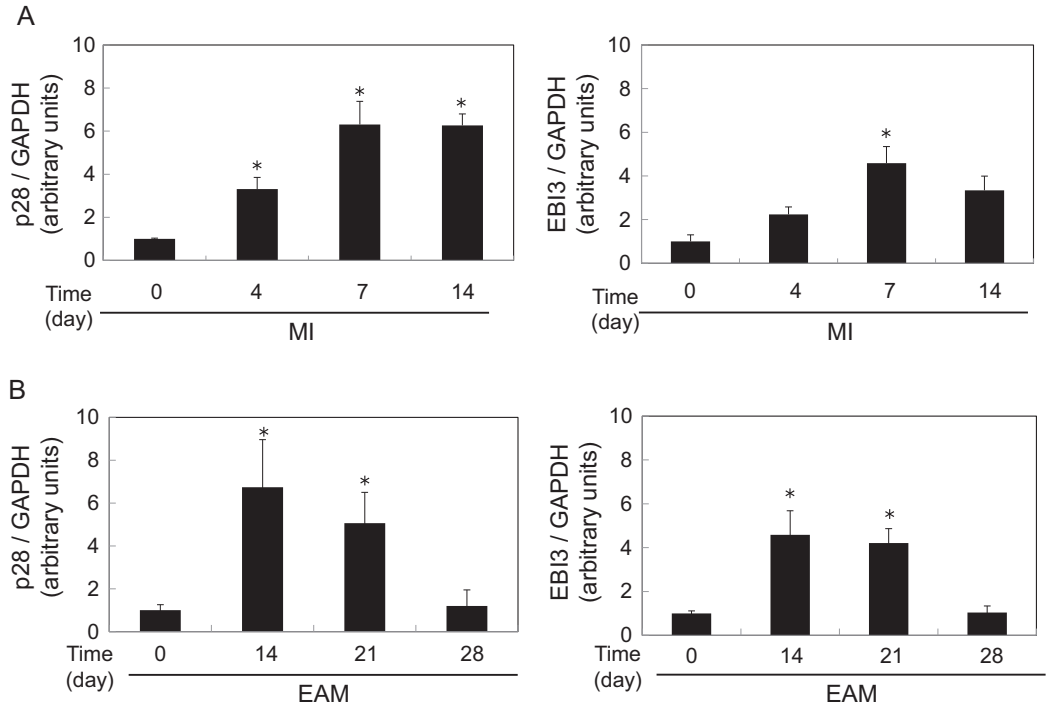

Fig. 2. Transcript expression of IL-27 was upregulated in murine hearts of cardiac injury models. The expression of p28 and EBI3, which are components of IL-27 cytokine, in MI hearts (A) and EAM hearts (B) were analyzed by quantitative RT-PCR. GAPDH was used as the internal control. Data were shown as mean ± S.E. (N = 3–8 for MI model, N = 6–8 for EAM model). *P < 0.05 by Steel's test versus day 0.

(MOI) of 50 for 24 h, and then cultured under the indicated conditions.

2.8. Statistical analysis

Statistical analysis was performed by unpaired *t*-test, ANOVA with post-hoc multiple comparison test or Steel's test. A value of less than 0.05 was considered significant. Data were shown as the mean value ± standard error (S.E.).

3. Result

3.1. IL-27 cytokine induced the expression of the EC specific markers in cardiac Sca-1+ cells

To address the effects of the IL-12 family cytokines on the differentiation of cardiac Sca-1+ cells, we first examined the expression of the receptors for these cytokines in cardiac Sca-1+ cells by RT-PCR (Fig. 1A). *IL-27Rα* mRNA was expressed in cardiac Sca-1+ cells, while the transcripts of *IL-12Rβ1*, *IL-12Rβ2*, and *IL-23R* were not detected. By immunofluorescent microscopic analyses, we have confirmed that more than 90% of cardiac Sca-1+ cells expressed IL-27Rα protein (Fig. 1B). Thus, we focused on IL-27 functions in further experiments of this study.

Previously, we have reported that the upregulation of IL-6 family cytokines is likely to induce EC differentiation of Sca-1+ cells after MI [7,8]. To explore the possibility that cardiac Sca-1+ cells could be stimulated with IL-27 under pathophysiological condition, we examined the gene expression profiles of *p28* and *EBI3*, which are components of IL-27, in murine hearts using two experimental disease models. Quantitative RT-PCR analyses revealed that both genes were significantly upregulated in post-infarct hearts (Fig. 2A). Moreover, the increase of these gene transcripts was also observed in EAM hearts (Fig. 2B).

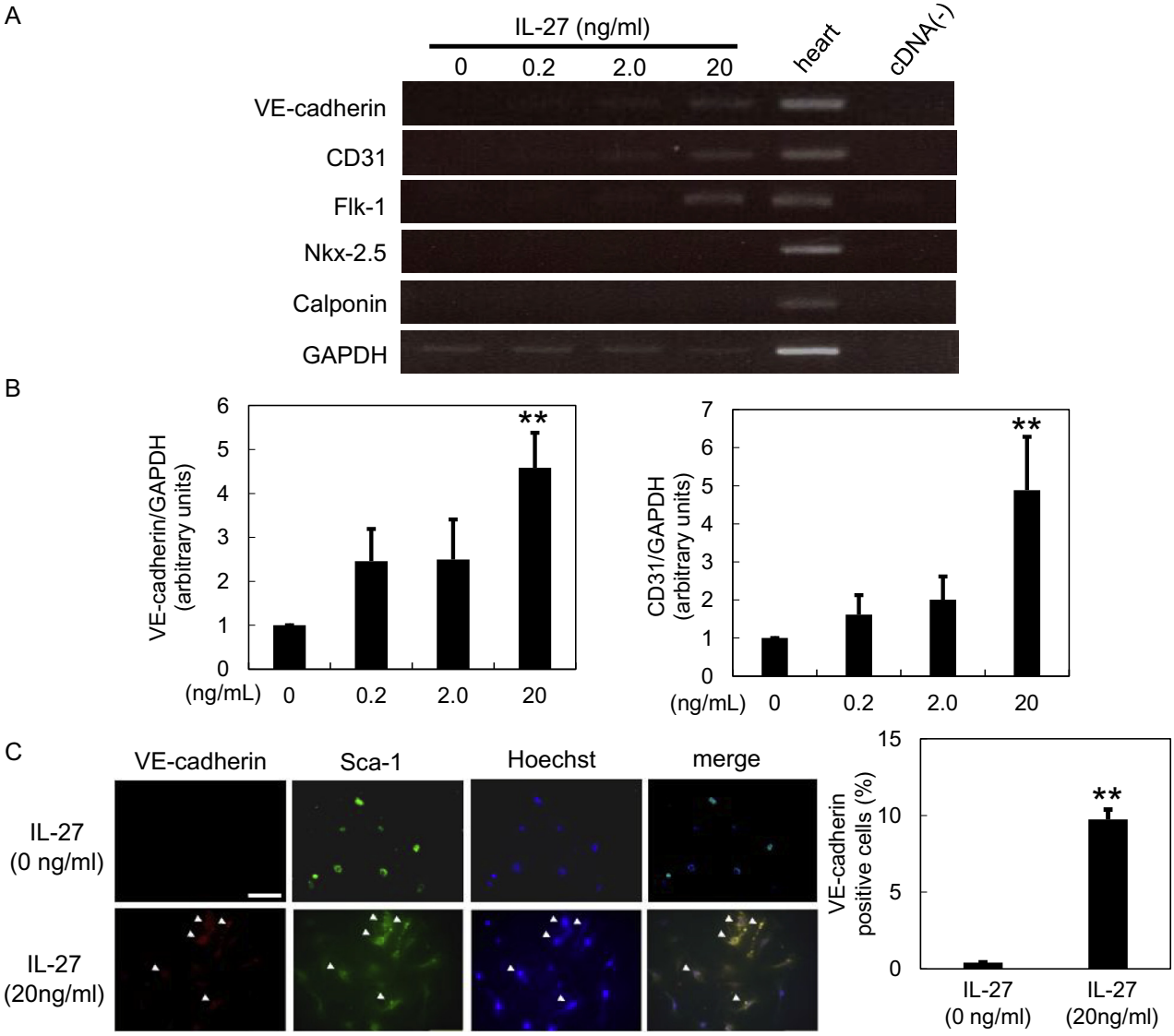

Fig. 3. IL-27 induces EC markers, but not cardiomyocyte marker and smooth muscle cell marker, in cardiac Sca-1+ cells. Cardiac Sca-1+ cells were cultured with the indicated concentrations of IL-27 for 14 days. (A and B) RT-PCR assay was performed for *CD31*, *VE-cadherin*, *Nkx-2.5*, *calponin* and *GAPDH*. Total RNA from murine hearts was used as a positive control. Representative data are shown. Experiments were repeated four times with similar results. Quantitative RT-PCR was performed for *CD31* and *VE-cadherin*. The expressions of these genes were normalized with that of *GAPDH*. Data are shown as mean ± S.E. from four independent experiments. $^{**}P < 0.01$ by ANOVA followed by Williams test versus 0 ng/ml. (C) Immunocytochemical analysis was performed with anti-VE-cadherin antibody. The images are representative of 75 fields from three independent cell cultures. Bar = 100 μm. VE-cadherin positive cells were quantitatively estimated. Data are shown as mean ± S.E. $^{**}P < 0.01$ by Student *t* test versus 0 ng/ml.

IL-27 transduces its signals via the heterodimer receptor complex composed of IL-27Rα and glycoprotein 130 (gp130). Previously, we have demonstrated that gp130 is expressed in cardiac Sca-1+ cells [7]. Therefore, we examined whether IL-27 induces the endothelial differentiation. Cardiac Sca-1+ cells were cultured with IL-27 for 14 days. RT-PCR analyses revealed that the expression of the endothelial marker genes, such as *VE-cadherin*, *CD31*, and *flk-1*, was upregulated, whereas the expression of cardiomyocyte or smooth muscle cell specific marker genes, Nkx-2.5 and *calponin*, was not detected in either the presence or absence of IL-27 (Fig. 3A). Quantitative RT-PCR analyses demonstrated that the expression of EC-specific markers, *VE-cadherin* and *CD31*, was upregulated in response to IL-27 (20 ng/ml) (VE-cadherin: 4.6-fold, CD31: 4.7-fold compared with control, $n = 4$) (Fig. 3B). Moreover, immunofluorescent microscopic analyses have revealed that about 10% of Sca-1+ cells, cultured with IL-27 for 14 days, were positively stained with anti-VE-cadherin antibody (Fig. 3C). These findings suggest that IL-27Rα functionally transduces their signals and that stimulation of IL-27Rα with IL-27 results in endothelial differentiation in cardiac Sca-1+ cells.

3.2. IL-27 activated STAT3/Pim-1 axis in cardiac Sca-1+ cells

Previously, we reported that STAT3/Pim-1 pathway plays a critical role in endothelial differentiation of cardiac Sca-1+ cells by IL-6 family cytokines both *in vitro* and *in vivo* [8]. To investigate the importance of STAT3/Pim-1 signaling in IL-27-mediated commitment of cardiac Sca-1+ cells to EC lineage, we examined whether IL-27 activates STAT3/Pim-1 axis in cardiac Sca-1+ cells.

Cardiac Sca-1+ cells were stimulated with IL-27 for the indicated time, and phosphorylation of STAT3 was analyzed by immunoblotting with anti-phospho-STAT3 antibody (Fig. 4A). STAT3 was prominently phosphorylated by IL-27, reaching a peak within 30 min of stimulation, and their activities were reduced to the basal level 60 min after stimulation, whereas the phosphorylation of ERK1/2 was not significantly enhanced. Moreover, STAT3 was phosphorylated by IL-27 in a dose-dependent manner (Fig. 4B).

Next, we examined whether IL-27 induces Pim-1 in cardiac Sca-1+ cells (Fig. 5A and B). Quantitative RT-PCR analysis confirmed that the expression of *Pim-1* transcript was significantly upregulated 30 min after IL-27 stimulation and returned to baseline within 3 h, followed by the reactivation afterward. The expression of *Pim-1* mRNA was enhanced in a dose-dependent manner. Western blot analyses confirmed that Pim-1 protein level was increased by IL-27 treatment.

Since Pim-1 kinase is a downstream target of STAT3 in cardiac Sca-1+ cells [8], we investigated whether STAT3 activation is required for Pim-1 induction by IL-27 (Fig. 6A and B). Adenoviral gene transfer of dominant-negative STAT3 (dn-STAT3) abrogated

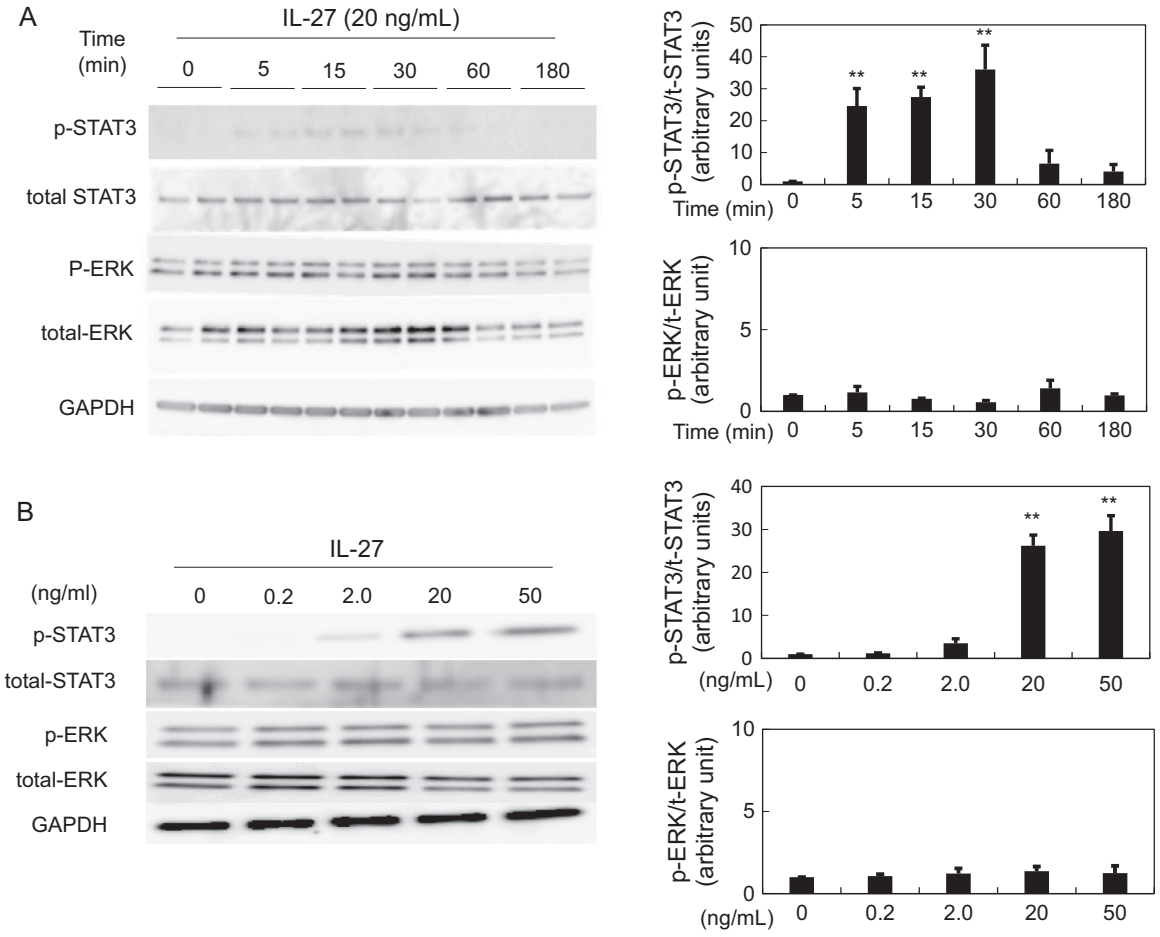

Fig. 4. IL-27 activates STAT3 in cardiac Sca-1+ cells. (A) Cardiac Sca-1+ cells were stimulated with IL-27 (20 ng/mL) for the indicated time. Cell lysates were immunoblotted with anti-p-STAT3 or anti-p-ERK antibody. Blots were reprobed with anti-STAT3 or anti-ERK antibody, respectively. Cell lysates were also blotted with anti-GAPDH antibody to show the equal amount loading. Representative data are shown in the left panel. Quantitative analyses by densitometry were shown in the right panel. Data are shown as mean ± S.E. from three independent experiments. $^{**}P < 0.01$ by ANOVA with Dunnet test versus 0 min. (B) Cells were stimulated with the indicated concentrations of IL-27 for 30 min. Phosphorylation of STAT3 and ERK was examined by immunoblot analyses, as described above. Representative data are shown in the left panel. Quantitative analyses by densitometry were shown in the right panel. Data are shown as mean ± S.E. from three independent experiments. $^{**}P < 0.01$ by ANOVA followed by Williams test versus 0 ng/ml.

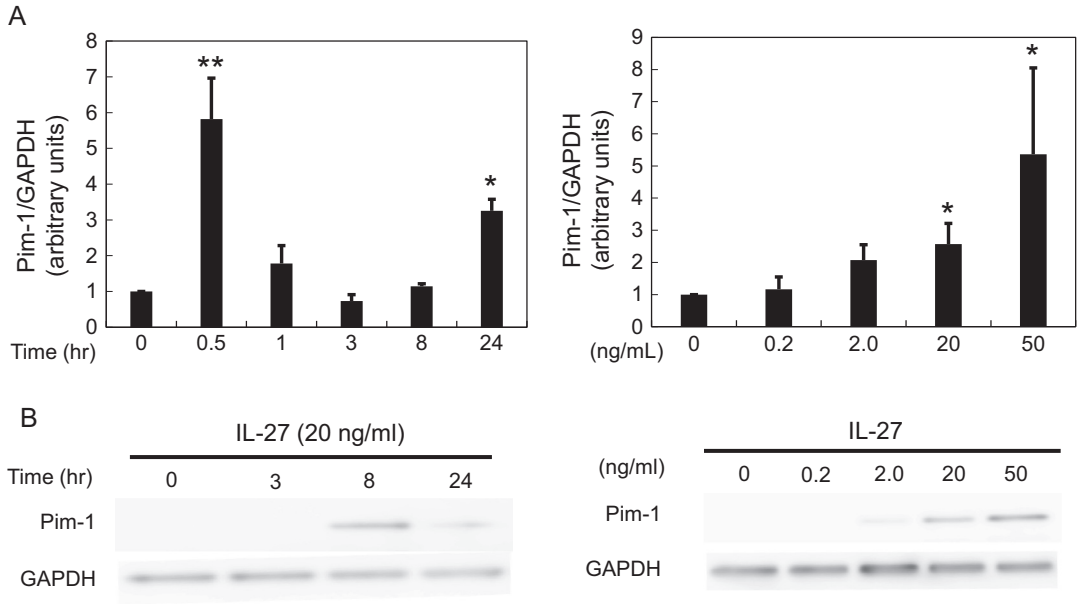

Fig. 5. IL-27 upregulates the expression of Pim-1 mRNA and protein. (A) Total RNA was prepared from cardiac Sca-1+ cells stimulated with IL-27. The *Pim-1* gene expression was analyzed by quantitative RT-PCR. The expression of *Pim-1* was normalized with that of *GAPDH*. Left panel, cells were stimulated with IL-27 (20 ng/ml) for the indicated time. Data are shown as mean ± S.E. $^{*}P < 0.05$, $^{**}P < 0.01$ by ANOVA with Dunnet test versus 0 h. Right panel, cells were stimulated with the various concentrations of IL-27 for 30 min. Data are shown as mean ± S.E. $^{*}P < 0.05$ by ANOVA followed by Williams test versus 0 ng/ml. (B) The Pim-1 protein expression was analyzed by immunoblotting. Left panel, cells were stimulated with IL-27 (20 ng/ml) for the indicated time. Right panel, cells were stimulated with the various concentrations of IL-27 for 8 h.

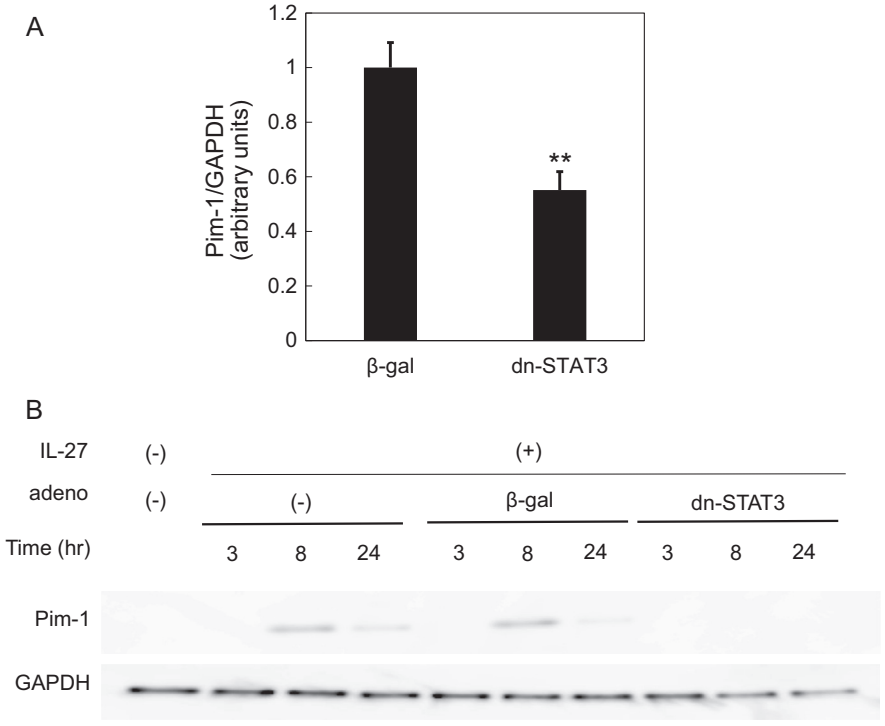

Fig. 6. STAT3 activation is required for IL-27-induced Pim-1 expression. Cardiac Sca-1+ cells were transfected with adenovirus vector expressing dominant negative STAT3 (dn-STAT3) or β-galactosidase (β-gal), as a control. (A) Cells were stimulated with IL-27 (20 ng/ml) for 30 min. *Pim-1* transcript was measured by quantitative RT-PCR. Data are shown as mean ± S.E. from four independent experiments. $^{**}P < 0.01$ by Welch's test. (B) Cells were incubated with or without IL-27 (20 ng/ml) for the indicated time. Pim-1 protein was evaluated by immunoblot analyses. Representative data are shown. Experiments were repeated three times with similar results.

IL-27-mediated induction of *Pim-1* mRNA and protein, while the upregulation of Pim-1 expression by IL-27 was observed in the cells expressing β-galactosidase (β-gal), a control. These findings indicate that STAT3 activation is necessary for Pim-1 induction by IL-27.

3.3. STAT3/Pim-1 axis is required for IL-27-mediated commitment of cardiac Sca-1+ cells to EC lineage

To examine whether Pim-1 activation is essential for the endothelial differentiation of cardiac Sca-1+ cells by IL-27, we

analyzed the effects of the inhibition of Pim-1 by adenoviral vectors expressing dominant negative Pim-1 (dn-Pim-1) (Fig. 7A and B). IL-27 failed to induce the endothelial differentiation in dn-Pim-1-expressing cells, whereas endothelial-specific genes were upregulated by IL-27 in the cells expressing β-gal. The inhibition of Pim-1 signaling did not influence the expression of cardiomyocyte or smooth muscle cell marker genes, *Nkx-2.5* and *calponin*. These data indicate that STAT3/Pim-1 plays functional roles in IL-27-induced ECs differentiation *in vitro*.

4. Discussion

In this study, we examined the biological effects of IL-27 on the transdifferentiation of Sca-1+ cardiac resident stem cells. Here, we showed that IL-27Rα was expressed in cardiac resident stem cells. Additionally, the expression of IL-27 transcript was upregulated in MI and EAM hearts. Importantly, the stimulation of IL-27Rα with IL-27 resulted in endothelial differentiation in cardiac resident stem cells. To our knowledge, this is the first demonstration that IL-27 exhibits its biological function in tissue resident stem cells.

WSX-1 was originally cloned as a novel orphan receptor, based on the similarity of cDNA sequence to the cytokine class I receptor family [11], and characterized as a component of IL-27R, IL-27Rα, afterward [15]. Interestingly, Northern blot analyses demonstrated that WSX-1 is highly expressed in the heart [11]; however, biological functions of IL-27 in the heart remain to be addressed, because IL-27 has been mainly considered as an immunoregulator. In this study, we discovered that cardiac Sca-1+ resident stem cells expressed IL-27Rα and that IL-27 transduced the differentiation signals in cardiac Sca-1+ cells. We also found that IL-27Rα was expressed in cardiac myocytes (data not shown). We will discuss the functional significances of IL-27Rα in cardiomyocytes elsewhere.

So far, it has been thought that hearts have the limited regenerative capacity, because cardiac myocytes cease to proliferate immediately after birth. However, recent studies identified several kinds of cardiac resident stem cells, such as Sca-1+ cells and c-kit+ cells [16,17]. These cells can differentiate into cardiac cells, including ECs, smooth muscle cells and cardiac myocytes, suggesting that cardiac resident stem cells can play crucial roles in cardiac repair/regeneration. Previously, we demonstrated that IL-6 family cytokines induces the endothelial differentiation of Sca-1+ cells through STAT3/Pim-1 pathway [8]. Here, similar to IL-6 family cytokines, IL-27 activated STAT3/Pim-1 pathway in Sca-1+ cardiac resident stem cells, leading to the transdifferentiation into the ECs. Importantly, the transplantation of cardiac Sca-1+ stem cells into myocardium attenuated adverse cardiac remodeling after myocardial injury, while cardiac Sca-1+ cells, whose STAT3/Pim-1 signaling pathway was inhibited, failed to exhibit cardioprotective effects [8]. Thus, the activation of STAT3/Pim-1 by IL-27 or other cytokines in cardiac Sca-1+ cells is likely to be a novel strategy that enhances cardiac repair/regeneration.

Here, we have demonstrated that *IL-27* transcript expression was elevated in murine MI hearts. Interestingly, the expression of IL-27 peaked from days 7 to 14 after MI., while IL-6 family cytokines, such as IL-11 and LIF, increased in the hearts immediately after MI [7], suggesting IL-27 might function as an activator of STAT3 in later phase of MI, compared with IL-6 family cytokines. Recently, it has been demonstrated that serum level of IL-27 is increased in the patients with acute myocardial infarction, compared with the control [18], proposing the clinical relevance of the importance of IL-27 in cardiovascular diseases.

The termination of inflammatory reactions is followed by tissue repair/regeneration. A number of studies have demonstrated that IL-27 exhibits immunoregulatory effects in experimental

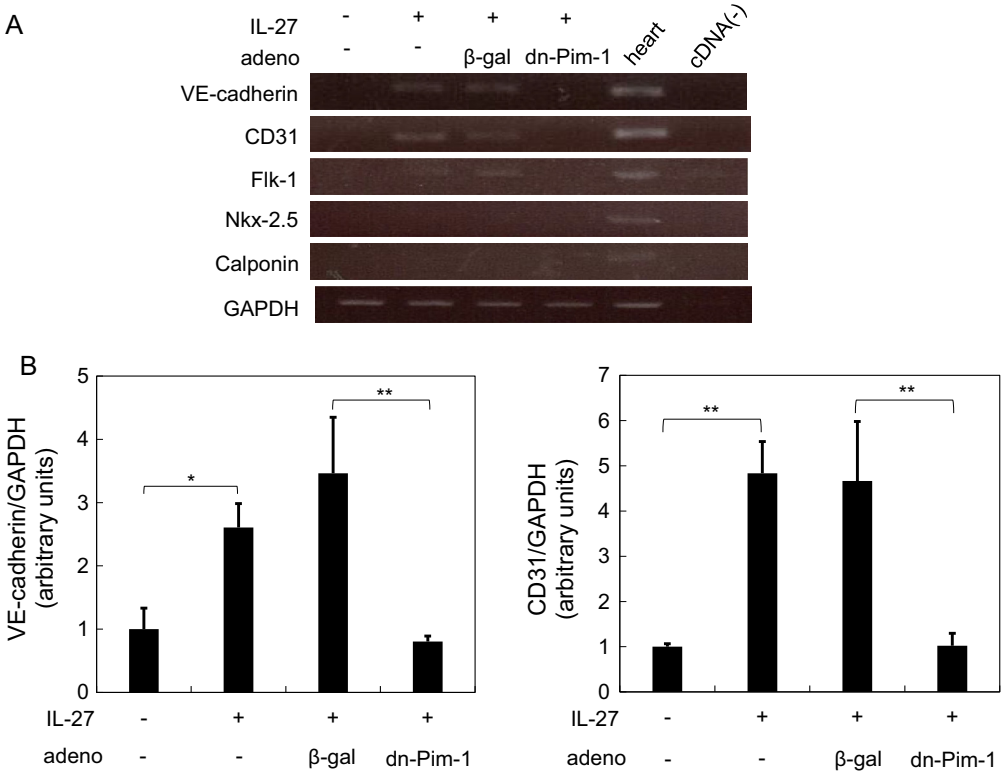

Fig. 7. Adenoviral overexpression of dominant negative Pim-1 abrogated IL-27-induced EC differentiation in cardiac Sca-1+ cells. Cardiac Sca-1+ cells were transfected with adenovirus vector expressing dominant negative Pim-1 (dn-Pim-1) or β-gal and incubated with or without IL-27 (20 ng/ml) for 14 days. (A) The effects of dn-Pim-1 on Sca-1+ cell differentiation into cardiac cells were analyzed by RT-PCR. (B) Quantitative RT-PCR was performed for *VE-cadherin* and *CD31*. The expressions of these genes were normalized with that of GAPDH. Data are shown as mean ± S.E. from four independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$ by one-way ANOVA followed by Tukey–Kramer test.

autoimmune diseases [19–24]. For example, IL-27 suppresses the inflammatory reaction in experimental autoimmune encephalomyelitis (EAE) [19,20]. IL-27R-deficient mice are susceptible to EAE with the increased Th17 cells. Similarly, the expression of IL-27 attenuates collagen-induced arthritis [21]. Interestingly, these experimental autoimmune models are self-limiting. The tissue damage, induced by inflammatory reaction, is repaired after the inflammatory process terminates. Considering the regenerative property of IL-27 on cardiac resident stem cells as a non-immune function, our data could propose a possibility that IL-27 contributes to the link between the tissue repair/regeneration and the termination of inflammatory reactions in hearts.

In conclusion, IL-27 induces the endothelial differentiation in cardiac resident stem cells through STAT3/Pim-1 signaling pathway. These findings suggest that IL-27/IL-27R system could be a therapeutic target that might link the regulation of inflammatory reaction with tissue repair/regeneration in cardiovascular diseases.

Disclosures

No conflicts of interest, financial or otherwise are declared by the author(s).

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References

[1] Taga T, Kishimoto T. Gp130 and the interleukin-6 family of cytokines. Annu. Rev. Immunol. 1997;15:797–819.
[2] Funamoto M, Fujio Y, Kunisada K, Negoro S, Tone E, Osugi T, et al. Signal transducer and activator of transcription 3 is required for glycoprotein 130-mediated induction of vascular endothelial growth factor in cardiac myocytes. J. Biol. Chem. 2000;275:10561–6.
[3] Osugi T, Oshima Y, Fujio Y, Funamoto M, Yamashita A, Negoro S, et al. Cardiac-specific activation of signal transducer and activator of transcription 3 promotes vascular formation in the heart. J. Biol. Chem. 2002;277:6676–81.
[4] Niu G, Wright KL, Huang M, Song L, Haura E, Turkson J, et al. Constitutive Stat3 activity up-regulates VEGF expression and tumor angiogenesis. Oncogene 2002;21:2000–8.
[5] Dankbar B, Padró T, Leo R, Feldmann B, Kropff M, Mesters RM, et al. Vascular endothelial growth factor and interleukin-6 in paracrine tumor-stromal cell interactions in multiple myeloma. Blood 2000;95:2630–6.
[6] Mohri T, Fujio Y, Maeda M, Ito T, Iwakura T, Oshima Y, et al. Leukemia inhibitory factor induces endothelial differentiation in cardiac stem cells. J. Biol. Chem. 2006;281:6442–7.
[7] Mohri T, Fujio Y, Obana M, Iwakura T, Matsuda K, Maeda M, et al. Signals through glycoprotein 130 regulate the endothelial differentiation of cardiac stem cells. Arterioscler. Thromb. Vasc. Biol. 2009;29:754–60.
[8] Iwakura T, Mohri T, Hamatani T, Obana M, Yamashita T, Maeda M, et al. STAT3/Pim-1 signaling pathway plays a crucial role in endothelial differentiation of cardiac resident Sca-1+ cells both in vitro and in vivo. J. Mol. Cell. Cardiol. 2011;51:207–14.
[9] Jones LL, Vignali DA. Molecular interactions within the IL-6/IL-12 cytokine/receptor superfamily. Immunol. Res. 2011;51:5–14.
[10] Vignali DA, Kuchroo VK. IL-12 family cytokines: immunological playmakers. Nat. Immunol. 2012;13:722–8.
[11] Sprecher CA, Grant FJ, Baumgartner JW, Presnell SR, Schrader SK, Yamagiwa T, et al. Cloning and characterization of a novel class I cytokine receptor. Biochem. Biophys. Res. Commun. 1998;246:82–90.
[12] Obana M, Maeda M, Takeda K, Hayama A, Mohri T, Yamashita T, et al. Therapeutic activation of signal transducer and activator of transcription 3 by interleukin-11 ameliorates cardiac fibrosis after myocardial infarction. Circulation 2010;121:684–91.
[13] Yamashita T, Iwakura T, Matsui K, Kawaguchi H, Obana M, Hayama A, et al. IL-6-mediated Th17 differentiation through RORγt is essential for the initiation of experimental autoimmune myocarditis. Cardiovasc. Res. 2011;91:640–8.
[14] Fujio Y, Nguyen T, Wencker D, Kitsis RN, Walsh K. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-reperfusion injury in mouse heart. Circulation 2000;101:660–7.
[15] Pflanz S, Hibbert L, Mattson J, Rosales R, Vaisberg E, Bazan JF, et al. WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. J. Immunol. 2004;172:2225–31.
[16] Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc. Natl. Acad. Sci. USA 2003;100:12313–8.
[17] Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003;114:763–76.
[18] Jafarzadeh A, Nemati M, Rezayati MT. Serum levels of interleukin (IL)-27 in patients with ischemic heart disease. Cytokine 2011;56:153–6.
[19] Fitzgerald DC, Zhang GX, El-Behi M, Fonseca-Kelly Z, Li H, Yu S, et al. Suppression of autoimmune inflammation of the central nervous system by interleukin 10 secreted by interleukin 27-stimulated T cells. Nat. Immunol. 2007;8:1372–9.
[20] Fitzgerald DC, Ciric B, Touil T, Harle H, Grammatikopolou J, Das Sarma J, et al. Suppressive effect of IL-27 on encephalitogenic Th17 cells and the effector phase of experimental autoimmune encephalomyelitis. J. Immunol. 2007;179:3268–75.
[21] Niedbala W, Cai B, Wei X, Patakas A, Leung BP, McInnes IB, et al. Interleukin 27 attenuates collagen-induced arthritis. Ann. Rheum. Dis. 2008;67:1474–9.
[22] Amadi-Obi A, Yu CR, Liu X, Mahdi RM, Clarke GL, Nussenblatt RB, et al. TH17 cells contribute to uveitis and scleritis and are expanded by IL-2 and inhibited by IL-27/STAT1. Nat. Med. 2007;13:711–8.
[23] Miyazaki Y, Shimanoe Y, Wang S, Yoshida H. Amelioration of delayed-type hypersensitivity responses by IL-27 administration. Biochem. Biophys. Res. Commun. 2008;373:397–402.
[24] Sugiyama N, Nakashima H, Yoshimura T, Sadanaga A, Shimizu S, Masutani K, et al. Amelioration of human lupus-like phenotypes in MRL/lpr mice by overexpression of interleukin 27 receptor alpha (WSX-1). Ann. Rheum. Dis. 2008;67:1461–7.